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A Microsatellite Linkage Map of Rainbow Trout (*Oncorhynchus mykiss*) Characterized by Large Sex-Specific Differences in Recombination Rates

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ABSTRACT

We constructed a genetic linkage map for a tetraploid derivative species, the rainbow trout (*Oncorhynchus mykiss*), using 191 microsatellite, 3 RAPD, 7 ESMP, and 7 allozyme markers in three backcross families. The linkage map consists of 29 linkage groups with potential arm displacements in the female map due to male-specific pseudolinkage arrangements. Synteny of duplicated microsatellite markers was used to identify and confirm some previously reported pseudolinkage arrangements based upon allozyme markers. Fifteen centromeric regions (20 chromosome arms) were identified with a half-tetrad analysis using gynogenetic diploids. Female map length is ~10 M, but this is a large underestimate as many genotyped segments remain unassigned at a LOD threshold of 3.0. Extreme differences in female:male map distances were observed (ratio F:M, 3.25:1). Females had much lower recombination rates (0.14:1) in telomeric regions than males, while recombination rates were much higher in females within regions proximal to the centromere (F:M, 10:1). Quadrivalent formations that appear almost exclusively in males are postulated to account for the observed differences.

FISH in the family Salmonidae are believed to be descended from a single tetraploid event estimated to have occurred 25–100 mya (Allendorf and Thorgaard 1984). This is supported by the fact that many homeologous chromosome arms still exchange chromatid segments as a result of quadrivalent formations during meiosis (Lee and Wright 1981; Wright *et al.* 1983; Allendorf and Thorgaard 1984). One unusual feature of this phenomenon is that it appears to be almost exclusive to males (Allendorf and Thorgaard 1984; Allendorf and Danzmann 1997). Furthermore, available allozyme evidence suggests that most chromosomal exchanges between homeologs are telomeric or subtelomeric as functionally duplicated genes map to these locations (Allendorf *et al.* 1986).

The mechanism ensuring duplication of some genes and diploidization of others may involve differential crossovers between homeologous chromosomes. Duplicated gene regions are preserved because of crossovers between homeologous chromosome segments distal to the centromere (Wright *et al.* 1983). Recombination likely retards the overall process of diploidization within telomeric segments (Allendorf *et al.* 1986). Regions proximal to the centromere are relatively immune to crossovers during quadrivalent formation, thus facilitating the diploidization of loci. Secondary tetrasomic segregation (crossover of chromosome arms in distal regions) as opposed to primary tetrasomic segregation (whole arm pairing and crossovers) may explain the mosaic nature of the salmonid genome (Allendorf and Danzmann 1997). Crossover events regulate the affinity of chromosome arms to one another and determine rates of allelic exchange among isoloci (paralogous loci resulting from genome duplications), thus influencing their rate of diploidization.

The construction of a genetic map is a first step toward understanding the mechanics of chromosomal interactions in polyploid organisms. The first comprehensive salmonid linkage map was based on 54 allozyme loci from several salmonid species and their hybrids (May and Johnson 1990). The utility of this map was limited, however, because only a few markers were characterized for any one species. Recently, genetic linkage maps based on a wide variety of new molecular markers have been constructed. In fish, most linkage maps have been constructed using randomly amplified polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs), or probes homologous to nuclear interspersed elements (Postlethwait *et al.* 1994; Wada *et al.* 1995; Kocher *et al.* 1998; Young *et al.* 1998). Although these markers may be applied in a rapid and

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cost-effective manner for a single pedigree, their use across different families is limited unless the markers are cloned and sequenced to establish homology (Knapik *et al.* 1998).

Microsatellite or simple sequence repeat (SSR) marker-type maps have been constructed in many organisms (Crawford *et al.* 1995; Jacob *et al.* 1995; Crooijmans *et al.* 1996; Dib *et al.* 1996; Dietrich *et al.* 1996; Kappes *et al.* 1997; de Gortari *et al.* 1998) and have been used to help locate genes for hereditary diseases and quantitative trait loci (QTL) controlling traits of economic importance (Andersson *et al.* 1994; Georges *et al.* 1995; Grobet *et al.* 1997; Knott *et al.* 1998). SSR markers are largely codominantly expressed, evenly distributed throughout the genome, and surveyed rapidly in many individuals using PCR techniques (Lee and Kocher 1996; Slettan *et al.* 1997; Knapik *et al.* 1998). Also, a large advantage of SSR markers is that they are hypervariable, which very often results in the detection of all four segregating chromosome regions (including null alleles) in the progeny, thus facilitating a direct comparison of sex-specific recombination rates within related full-sib genomic backgrounds. Linkage maps based on microsatellites in fish are few with a notable exception being the genetic linkage maps for zebrafish and tilapia (Knapik *et al.* 1998; Kocher *at al.* 1998).

In this article, we report the first comprehensive male and female-specific SSR linkage maps in rainbow trout. In salmonids, microsatellite markers are often conserved among closely related species (Morris *et al.* 1996; Presa and Guyomard 1996; Sakamoto *et al.* 1996). Thus, we provide information on where SSR markers from Atlantic salmon (*Salmo salar*), brown trout (*S. trutta*), chinook salmon (*Oncorhynchus tshawytscha*), chum salmon (*O. keta*), cutthroat trout (*O. clarkii*), marble trout (*S. marmoratus*), pink salmon (*O. gorbusha*), and sockeye salmon (*O. nerka*) are located within the rainbow trout map. Evidence for large sex-specific differences in recombination rates and large intrachromosomal differences in recombination rates between families is given. Large differences in recombination rates are expected to occur between the sexes in salmonids due to the differential sex-specific alignment of chromosomes during meiosis (Lee and Wright 1981). Also, the comparative mapping of duplicated SSR markers is used to help identify and verify observed residual tetrasomic (pseudolinkage) arrangements in the male parents used for gene mapping.

MATERIALS AND METHODS

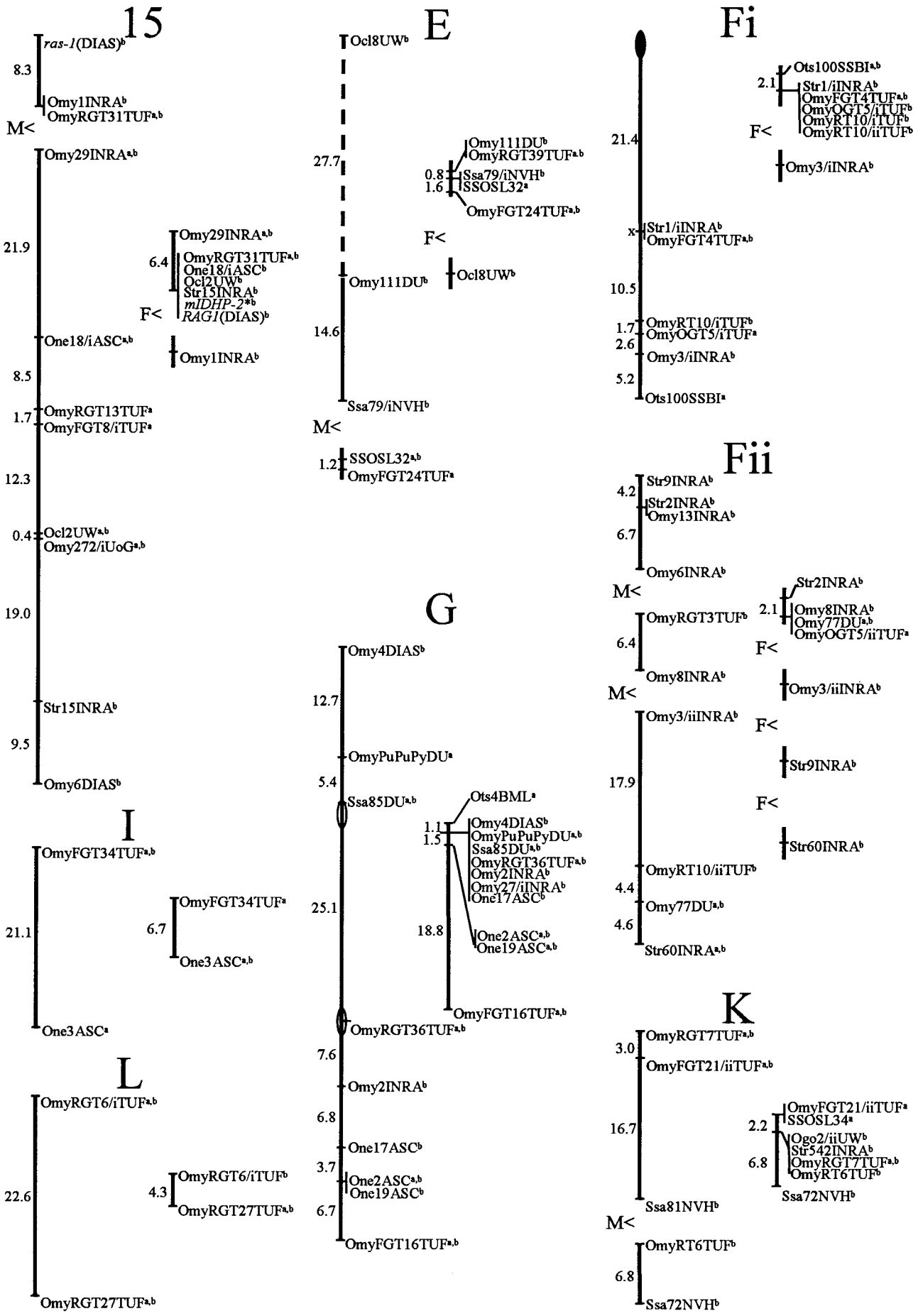
Rainbow trout backcross families: We used three backcross families (lot 25, $n = 48$; lot 41, $n = 48$; and lot 44, $n = 90$) previously utilized to detect QTL for upper temperature tolerance (Jackson *et al.* 1998; Danzmann *et al.* 1999) and spawning time (Sakamoto *et al.* 1999). Most of the mapping data was assimilated from only two of these families (lots 25 and 44), and additional genotypic data were collected from lot 41 when the parents for the other two lots were uninformative. All the families used F_1 hybrid males (derived from strains selected for the opposite phenotypic traits being studied) and pure strain females (see Jackson *et al.* 1998 for details).

Microsatellite analysis: Specific protocols used to analyze SSR polymorphism varied among the labs of the contributing authors. Generally, genomic DNAs were extracted from muscle, liver, or gill tissue from the backcross progeny and their parents (*e.g.*, Bardakci and Skibinski 1994) and PCR was performed in a 11-μl reaction volume using 96-well microtiter plates following protocols similar to those outlined in Sakamoto *et al.* (1996) using primers end-labeled with [γ-^{33}P]ATP or fluorochromes or with direct incorporation of a fluorochrome during the PCR reaction. PCR programs used to amplify SSR DNA were similar to the following: initial denaturation at 95° for 2 min, followed by 35 cycles consisting of 1 min at the annealing temperature, 1 min at 72°, 30 sec at 95°, and a final extension of 3 min at 72°. PCR products were separated in a 6% polyacrylamide-7-m urea gel and the resulting DNA fragments were either visualized following autoradiography (using Kodak Biomax MR film) or with a fluorescence imaging system (*e.g.*, Hitachi FMBIOII).

RAPD markers: Primers for generating RAPD were obtained from the University of British Columbia (kit 1). Markers were designated according to the size of the polymorphic DNA fragment detected with a given primer. The size of the DNA fragment is indicated in kilobases after the hyphen following the primer name (*e.g.*, UBC100-2.13).

Genes: Functional genes were located on the map by using

Figure 1.—Comparative female (left) and male (right) linkage map of rainbow trout based primarily upon SSR markers. Numbered linkage groups correspond to those of May and Johnson (1990) based upon syntenic linkages with an identified allozyme marker. The mapping family used to obtain the segregation data is indicated by a superscript following the marker name (a, lot 44; b, lot 25; c, lot 41). Possible linkage relationships (LOD $>$ 2.0) for three markers are indicated with a dashed line, while all other markers are joined at a LOD $>$ 3.0. Markers linked in the male map but unlinked in the female map (LOD $<$ 3.0) are indicated by M$<$, while markers linked in the female map but unlinked in the male map (LOD $<$ 3.0) are indicated by F$<$. The estimated position of centromeres on linkage groups was obtained from a half-tetrad analysis using two or more markers. The marker closest to the centromere is used to obtain a gene-centromere estimate and is indicated with an "x." Two possible centromeric positions on linkage group G were ascertained that were incompatible with recombination estimates between the adjacent markers. The positioning of certain markers in the male map cannot be ascertained since segregation data was not obtained for the complete set of markers in both mapping families. For example, the positioning of Ots100SSBI in Fi may be on either side of the central cluster. Similarly, Ssa72NVH may be on either side of the central cluster in K. Other linkage groups where the positioning of markers derived from the two mapping families is uncertain in males with respect to anchor loci are: A, J, Oi, Oii, and Q. One inversion in map positions within the female map was noted on linkage group 5 (One18/iiASC and OmyFGT8/iiTUF show an inversion in lot 25).


15
ras-1(DIAS)b
8.3
Omy1INRAb
OmyRGT31TUFa,b
M<
Omy29INRAa,b
21.9
Omy29INRAa,b
6.4
OmyRGT31TUFa,b
One18/iASCb
Ocl2UWb
Str15INRAb
mIDHP-2*b
F<
RAG1(DIAS)b
One18/iASCa,b
Omy1INRAb
8.5
OmyRGT13TUFa
1.7
OmyFGT8/iTUFa
12.3
Ocl2UWa,b
0.4
Omy272/iUoGa,b
19.0
Str15INRAb
9.5
Omy6DIASb
I
OmyFGT34TUFa,b
21.1
OmyFGT34TUFa
6.7
One3ASCa,b
One3ASCa
L
OmyRGT6/iTUFa,b
22.6
OmyRGT6/iTUFb
4.3
OmyRGT27TUFa,b
OmyRGT27TUFa,b
E
Ocl8UWb
27.7
Omy111DUb
OmyRGT39TUFa,b
0.8
Ssa79/iNVHb
1.6
SSOSL32a
OmyFGT24TUFa,b
F<
Omy111DUb
Ocl8UWb
14.6
Ssa79/iNVHb
M<
1.2
SSOSL32a,b
OmyFGT24TUFa
G
Omy4DIASb
12.7
OmyPuPuPyDUa
5.4
Ssa85DUa,b
Ots4BMLa
1.1
Omy4DIASb
1.5
OmyPuPuPyDUa,b
Ssa85DUa,b
OmyRGT36TUFa,b
Omy2INRAb
Omy27/iINRAb
One17ASCb
25.1
18.8
One2ASCa,b
One19ASCa,b
OmyFGT16TUFa,b
OmyRGT36TUFa,b
7.6
Omy2INRAb
6.8
One17ASCb
3.7
One2ASCa,b
One19ASCb
6.7
OmyFGT16TUFa,b
Fi
Ots100SSBIa,b
2.1
Str1/iINRAb
OmyFGT4TUFa,b
OmyOGT5/iTUFb
OmyRT10/iTUFb
F<
OmyRT10/iiTUFb
21.4
Omy3/iINRAb
x-
Str1/iINRAb
OmyFGT4TUFa,b
10.5
OmyRT10/iTUFb
1.7
OmyOGT5/iTUFa
2.6
Omy3/iINRAb
5.2
Ots100SSBIa
Fii
Str9INRAb
4.2
Str2INRAb
Omy13INRAb
6.7
Omy6INRAb
M<
Str2INRAb
2.1
Omy8INRAb
Omy77DUa,b
OmyOGT5/iiTUFa
OmyRGT3TUFb
6.4
F<
Omy8INRAb
Omy3/iiINRAb
M<
Omy3/iiINRAb
F<
Str9INRAb
17.9
F<
Str60INRAb
OmyRT10/iiTUFb
4.4
Omy77DUa,b
4.6
Str60INRAa,b
K
OmyRGT7TUFa,b
3.0
OmyFGT21/iiTUFa,b
OmyFGT21/iiTUFa
2.2
SSOSL34a
16.7
Ogo2/iiUWb
Str542INRAb
6.8
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6.8
Ssa72NVHb

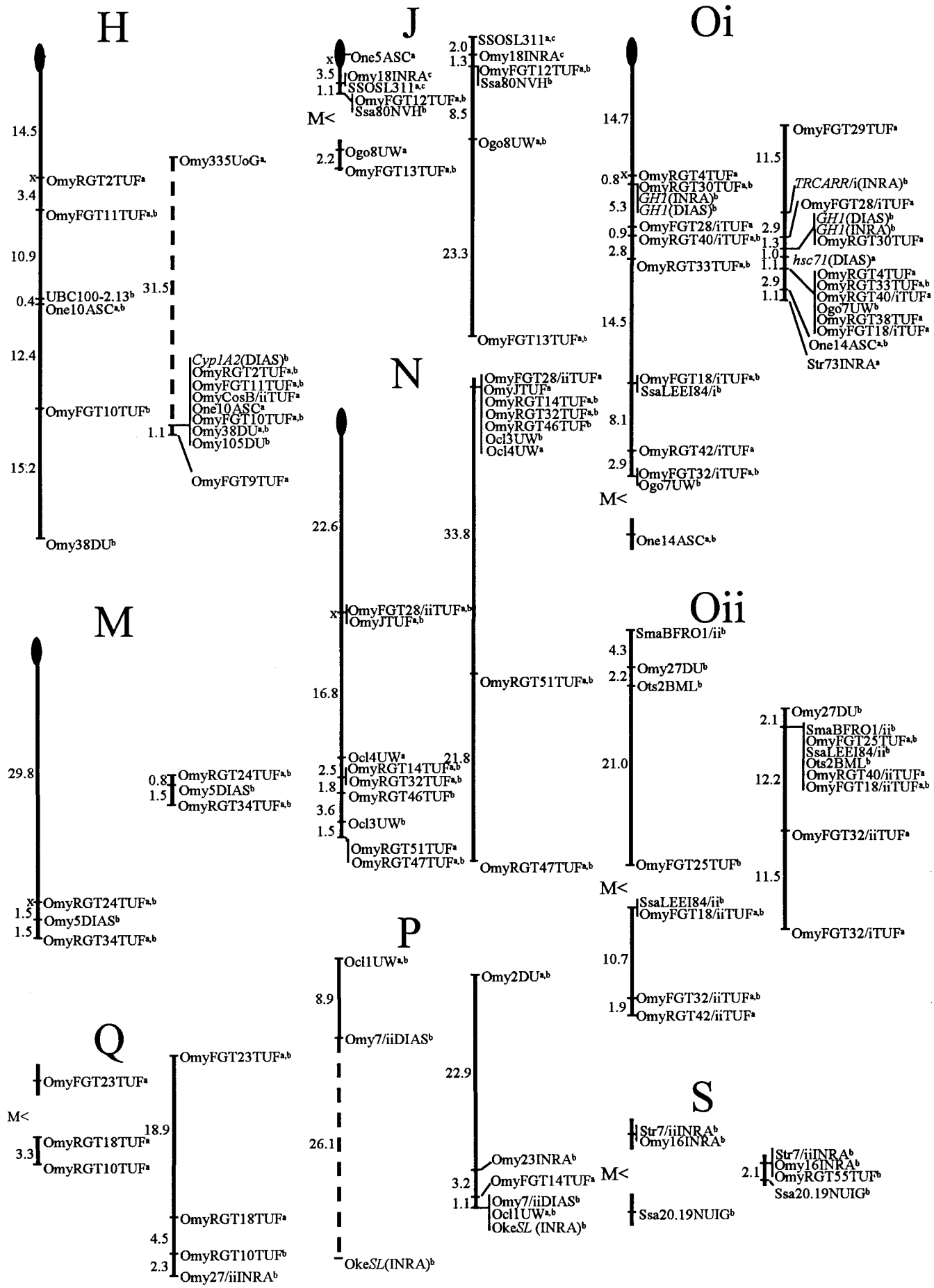

Figure 1.—*Continued.*

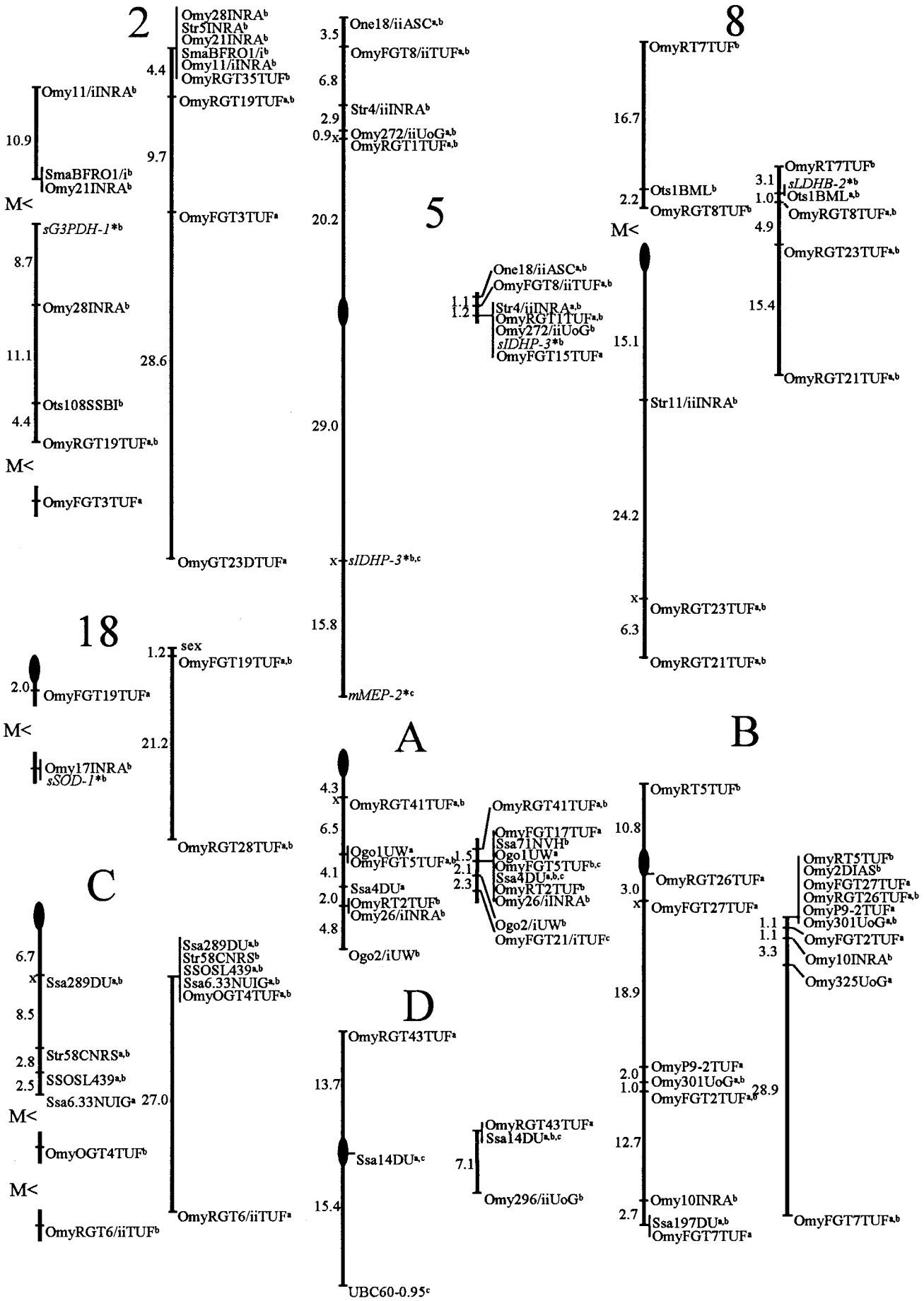

Figure 1.—*Continued.*

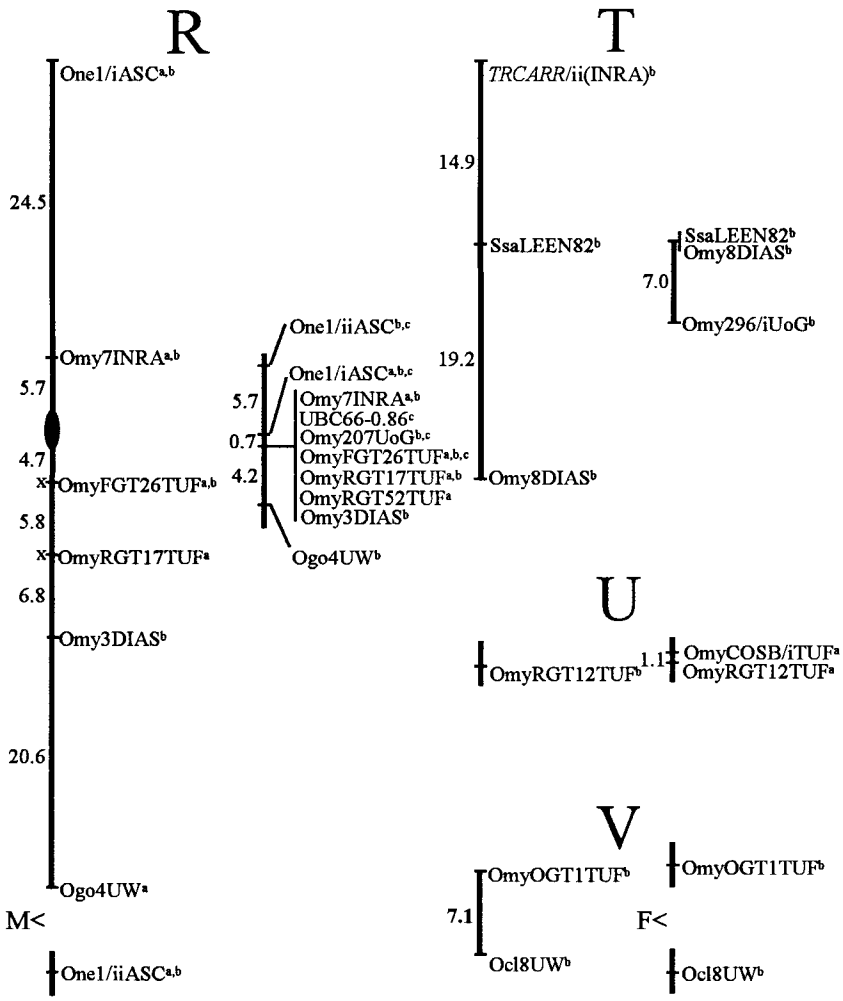

Figure 1.—*Continued.*

either allozyme polymorphisms or expressed sequence marker polymorphisms (ESMPs). ESMPs were identified by single nucleotide polymorphism (SNP) markers or by using SSR markers localized within known flanking or intron regions of functional genes. SNP markers were generated by designing primers to amplify across intron/exon junctions. The resulting PCR fragments were verified by sequencing and subjected to random restriction enzyme digests to detect intron polymorphisms. ESMPs are indicated in italics in Figure 1 according to their published gene designations followed by a lab acronym (Table 1) given in parentheses indicating the origin of the SNP or primer design. The ESMPs reported are *CYP1A2* (cytochrome P450, subfamily I, polypeptide 2), *GH1* (growth hormone 1), *hsc71* (71 kD heat shock cognate protein), *RAG1* (recombination activating protein I), *ras-1* (ras protein activator), *SL* (somatolactin gene), and *TRCARR* (trout red cell arrestin gene). If a primer/SNP variant was designed from a species other than rainbow trout and the variant detected was not confirmed by sequence homology with rainbow trout, the species origin for ESMP is indicated as a prefix to the gene name. Seven allozyme loci (glyceraldehyde-3-phosphate dehydrogenase, *G3PDH-1**, E.C. 1.1.1.8; *N*-acetyl-β-glucosaminidase, *bGLUA-2**, E.C. 3.2.1.30; isocitrate dehydrogenase ($NADP^+$), *mIDHP-2**, *sIDHP-3**, E.C. 1.1.1.42; l-lactate dehydrogenase, *sLDHB-2**, E.C. 1.1.1.27; malic enzyme ($NADP^+$), *mMEP-2**, E.C. 1.1.1.40; and superoxide dismutase, *sSOD-1**, E.C. 1.15.1.1) were used to assign microsatellite linkage groups to the composite salmonid linkage map published by May and Johnson (1990). Allozymes are indicated in italics in Figure 1 according to their published gene designations.

Gene-centromere map distances: Three female progeny from lot 44 were used as gynogenetic donor females in the assessment of gene-centromere distances of selected markers. Methods of analysis follow those outlined in Allendorf *et al.* (1986), Allendorf and Leary (1984), and Thorgaard *et al.* (1983) where gene-centromere distances are calculated as $y/2$, where y is the number of heterozygous recombinants scored/total sample size. Individual markers were first assessed for deviation from Mendelian expectations. At least two markers per linkage arm were analyzed to determine centromere-telomere orientation. The gene-centromere distance of the marker most proximal to the centromere was used in reporting map distances. The addition of more distal markers was made by joining adjacent marker positions by their estimated pairwise recombination distances (Θ).

Genetic nomenclature: We have followed the convention proposed by Jackson *et al.* (1998) for the naming of SSR markers. The designation begins with a three-letter acronym (for example, Omy = *O. mykiss*) followed by a lab-specific designation for each marker and a suffix acronym describing the lab of origin for the primers (see Table 1). When a primer set has previously been published using a lab designation, we have adopted the format of the published primer. Reference to the lab acronym and published sources are given in Table 1. Previously published primers with the SSOSL designation (Slettan *et al.* 1997) refer to primers developed from Atlantic salmon.

Linkage groups with allozyme markers described by May and Johnson (1990) are identified with the Arabic numeral designations given by these authors. In the absence of informa-

TABLE 1

Sources of the SSR primers used in this study

Species abbreviation	Common name	Scientific name
Ocl	Cutthroat trout	(*O. clarki*)
Ogo	Pink salmon	(*O. gorbuscha*)
Oke	Chum salmon	(*O. keta*)
Omy	Rainbow trout	(*O. mykiss*)
One	Sockeye salmon	(*O. nerka*)
Ots	Chinook salmon	(*O. tshawytscha*)
Sma	Marble trout	(*S. marmoratus*)
Ssa	Atlantic salmon	(*S. salar*)
Str	Brown trout	(*Salmo trutta*)

Laboratory abbreviation[a]	Official name and corresponding author
ASC	Alaska Science Center (USA), Scribner *et al.* (1996)
BFRO	University of Ljubljana (Slovenia), Snoj *et al.* (1997)
BML	Bodega Marine Laboratory, University of California (Davis), Banks *et al.* (1999)
CNRS	Centre National de Recherche Scientifique (France), Chantal Poteaux
DIAS	Danish Institute of Agricultural Science, Tjele (Denmark), L-E. Holm, Holm and Brusgaard (1998)
DU	Dalhousie University, Halifax, Nova Scotia (Canada), J. M. Wright; Morris *et al.* (1996)
INRA	Institut National de la Recherche Agronomique (Jouy-en-Josas, France), K. Gharbi and R. Guyomard
LEE	National Fish Health Research Laboratory (Leetown, West Virginia), W. B. Schill and R. L. Walker
NVH	Norwegian College of Veterinary Medicine (Oslo, Norway), B. Hoyheim
OSL	Norwegian College of Veterinary Medicine (Norway), Slettan *et al.* (1997)
NUIG	National University of Ireland (Galway), R. Powell
SSBI	SeaStar Biotech Incorporated (Victoria, British Columbia, Canada), Nelson and Beacham (1998)
TUF	Tokyo University of Fisheries (Japan), T. Sakamoto and N. Okamoto; Sakamoto *et al.* (1996)
UoG	University of Guelph (Canada), R. G. Danzmann and M. M. Ferguson; Jackson *et al.* (1998)
UW	University of Washington (USA), Olsen *et al.* (1998); Condrey and Bentzen (1998)

The acronym prefix for the species of origin with respect to primer design is indicated in the first part of the table.

[a] The source of the primer design is indicated by the lab of origin, which is listed in the second half of the table. Primers are identified according to their lab of origin by the acronym that appears as a suffix or with the primer name given throughout the text. Readers are referred to the publication(s) listed or the lab investigators listed as a source for information on primer sequences.

tion on segregating allozyme markers, we have tentatively identified new linkage groups alphabetically. Duplicated markers located on different linkage groups suggest that such chromosomes share homeology to one another and may potentially be recognized as new pseudolinkage groups. Such homeologous groups are designated with a lowercase i or ii following the alphabetic assignment of the linkage group. Similarly, duplicated SSR markers that are detected with a single pair of primers are indicated by a forward slash and a lowercase i or ii designation to denote each separate locus (*e.g.*, the Omy272-UoG primer set amplifies both the Omy272/iUoG and Omy272/iiUoG loci). Pseudolinkage is recognized as the generation of an apparent excess of recombinant genotypes following meiosis when the phase of the duplicated alleles is known. This occurs in male salmonids when homeologous chromosomes combine in multivalent formations during meiosis I (Wright *et al.* 1983). Since homeologous chromosome arms may not form multivalents during meiosis I, it is important to recognize that duplicated markers may not necessarily be pseudolinked. It should also be noted that the pseudolinkage groups described in this article may not contain complete information on the homeologous chromosome arms involved in multivalent pairings. Due to the nature of Robertsonian translocations in these fish, metacentric chromosomes may in fact be composed of two different homeologous acrocentric sets. Pseudolinkage within one of the ancestral homelogous sets may be detected with the microsatellites we have used, yet go undetected for the other homeologous group. It should be recognized that a revision of linkage group nomenclature for salmonid fishes will be necessary in the future as more complete information on linkage group syntenies is collected among species.

Linkage analysis: Linear assignments of markers within linkage groups were aided by the program MAPORD (R. G. Danz-

mann, unpublished results).[1] Map distances were assigned using observed recombination estimates between adjacent markers as salmonids have almost complete interference during meiosis (Thorgaard *et al.* 1983). When possible, map construction involved using LOD adjusted recombination estimates between anchor markers (*i.e.*, segregation data obtained from all mapping families). Map distances for solo markers (markers segregating in a subset of the mapping families) located within anchor marker intervals were adjusted using the LOD weighted recombination estimates between anchor markers with MAPDIS (R. G. Danzmann, unpublished results).[1] For a family with lower-than-average recombination, the map distance will be adjusted upward while a family with higher-than-average recombination will have the map distances adjusted downward. For placing markers on the map when they are located outside of an anchor marker interval (*i.e.*, at the ends of the linkage group), two approaches can be adopted: the observed recombination estimate (between the marker and the nearest anchor marker) may be used, or the LOD weighted recombination estimate of the nearest anchor pair may be used to readjust the map distance of the marker. The latter approach was used in constructing the female map. Thus, map distances were increased for terminal markers (*i.e.*, close to the centromere or telomere and outside of a common anchor interval) if they were derived from a family with lower-than-average recombination levels and were decreased if the segregation data was obtained from a family with higher-than-average recombination levels. In cases where anchor markers were not present in a given linkage group, the unadjusted recombination estimates obtained from each respective family were used as the map interval estimate.

Map construction was not averaged across the sexes due to the large differences in recombination rate detected between the sexes (Johnson *et al.* 1987; this study). Thus, sex-specific linkage maps were generated. Pairwise recombination estimates used as input into MAPORD and MAPDIS were obtained with LINKMFE4 (R. G. Danzmann, unpublished results),[1] which tests for independent segregation of male- and female-specific alleles across marker regions (*i.e.*, fully outcrossed genomes). Estimates of the differences in sex-specific recombination rates along chromosome intervals were conducted using the program RECOMDIF (R. G. Danzmann, unpublished results).[1]

RESULTS

Microsatellite linkage map: We constructed a genetic linkage map for rainbow trout (*O. mykiss*) with 209 markers composed of 191 SSR, 7 allozyme, 3 RAPD, and 7 ESMPs (one showing duplicate gene expression) in three backcross families. In addition segregation data were obtained for 6 SSR markers (Ogo3UW, Omy1D-IAS, Omy5/iINRA, Ssa79/iiNVH, Omy4/iINRA, and Omy4/iiINRA) in lot 25 and 6 SSR markers (OmyFGT6TUF, OmyFGT20TUF, OmyRGT15/iTUF, OmyRGT15/iiTUF, OmyRGT53TUF, and One11ASC) in lot 44, which remain unlinked to the other markers tested.

We detected 29 linkage groups using segregation data obtained from female parents that span ~10 M. An additional 3.5 M was detected using the segregation data from males for markers that were not polymorphic in females, but these distances may be biased by their relative chromosomal position (see below). A large number of markers that are assigned to the male map remain unassigned to the female map (Figure 1). Since the female map is relatively incomplete and female recombination rates are much higher than those observed in males, these markers may reside in the same linkage groups as described for males. Alternatively, these markers may also identify pseudolinkage groups that are only detected in males (see below). There are currently 49 unlinked segments in the female map at a specified LOD of 3.0 (and 47 segments at a LOD of 2.0; Figure 1). On the basis of the results of seven allozyme markers and the sex-determining locus, it is possible to identify five linkage groups (2, 5, 8, 15, and 18) reported by May and Johnson (1990). Two of these linkage groups (5 and 15) belong to pseudolinkage group V.

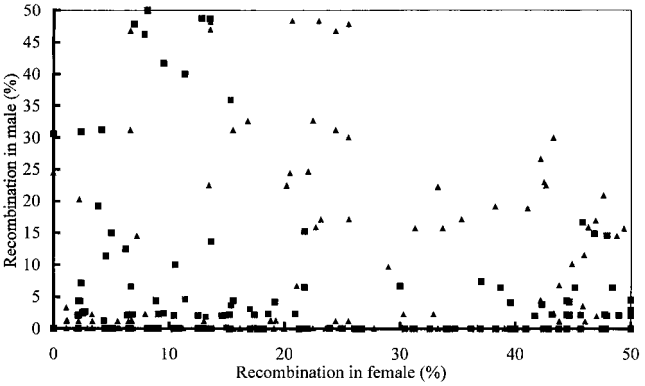

Figure 2.—Differences in male and female recombination among adjacently paired markers on all the linkage groups in rainbow trout (squares, lot 44; triangles, lot 25).

Differences in recombination rate between males and females: The sexes show substantial differences in recombination rate for the same pairs of linked markers. The ratio of female:male recombination rates among all adjacent markers is 3.25:1. Individual pairwise female:male recombination differences varied from infinity to 0.00 (Figure 2). This comparison includes all linkage groups detected in females except 18, S, U, and V. Female recombination rates exceeded male recombination rates for all homologous regions compared except those on linkage groups J, Q, and one segment on 8. Female recombination rates around the centromere were much higher than those of males. Conversely, male recombination rates appeared to be higher in telomeric regions. For example, male map distances between the two terminal markers on linkage groups 8, B, G, and N are substantially greater than those of the female (Table 2). Terminal markers in these linkage groups (OmyFGT7TUF in B, OmyFGT16TUF in G, and OmyFGT47TUF/OmyFGT51TUF in N) have estimated gene-centromere distances of 49.6 (G)–50 (B and N; P. Howard, unpublished data), confirming their telomeric location based on additive linkage distances in the female map.

[1]Software may be obtained from the following web address: http://www.uoguelph.ca/~rdanzman.

TABLE 2

Female:male recombination differences along several chromosome arms >40 cM in length and possessing an identified centromere

Family	Marker 1	Marker 2	Ratio F:M[a]	MapDis[b]	Sig.[c]
		Linkage group 8			
Lot 25	OmyRGT23TUF	OmyRGT21TUF	0.200	42.4	NS
Lot 44	OmyRGT23TUF	OmyRGT21TUF	0.500		NS
		Linkage group B			
Lot 44	OmyRT5TUF	OmyRGT26TUF	UnDef	5.4	$P < 0.001$
Lot 44	OmyRGT26TUF	OmyFGT27TUF	UnDef	12.3	NS
Lot 44	OmyFGT27TUF	OmyP9-2TUF	UnDef	23.2	$P < 0.001$
Lot 44	OmyP9-2TUF	Omy301UoG	UnDef	33.7	NS
Lot 44	Omy301UoG	OmyFGT2TUF	2.01	35.2	NS
Lot 44	OmyFGT2TUF	OmyFGT7TUF	0.50	43.4	$P < 0.05$
		Linkage group G			
Lot 44	OmyPuPuPyDU	Ssa85DU	UnDef	15.4	$P < 0.01$
Lot 44	Ssa85DU	OmyRGT36TUF	UnDef	30.6	$P < 0.001$
Lot 25	Ssa85DU	OmyRGT36TUF	UnDef		$P < 0.001$
Lot 25	OmyRGT36TUF	Omy2INRA	UnDef	46.9	$P < 0.05$
Lot 25	Omy2INRA	One17ASC	UnDef	54.1	$P < 0.05$
Lot 25	One17ASC	One2ASC	1.00	59.3	NS
Lot 25	One2ASC	OmyFGT16TUF	0.50	64.5	NS
Lot 44	One2ASC	OmyFGT16TUF	0.50		NS
		Linkage group H			
Lot 44	OmyFGT2TUF	OmyFGT11TUF	UnDef	16.2	NS
Lot 44	OmyFGT11TUF	One10ASC	UnDef	23.5	$P < 0.001$
Lot 25	OmyFGT11TUF	OmyFGT10TUF	UnDef	29.8	$P < 0.005$
Lot 25	OmyFGT10TUF	Omy38DU	UnDef	49.2	$P < 0.005$
		Linkage group N			
Lot 44	OmyFGT28/iiTUF	Ocl4UW	13.05	31.0	$P < 0.001$
Lot 44	Ocl4UW	OmyRGT14TUF	UnDef	40.7	NS
Lot 25	OmyRGT14TUF	OmyRGT46TUF	UnDef	42.8	NS
Lot 44	OmyRGT14TUF	OmyRGT51TUF	0.21	45.4	$P < 0.001$
Lot 25	OmyRGT46TUF	Ocl3UW	UnDef	45.5	NS
Lot 25	Ocl3UW	OmyRGT47TUF	0.14	48.0	$P < 0.001$
Lot 44	OmyRGT51TUF	OmyRGT47TUF	0.00	48.8	$P < 0.001$
		Linkage group Oi			
Lot 44	OmyRGT4TUF	OmyRGT40/iTUF	UnDef	18.2	$P < 0.001$
Lot 25	*GH1*(DIAS)	OmyRGT33TUF	UnDef	18.3	$P < 0.05$
Lot 44	OmyRGT40/iTUF	OmyFGT18/iTUF	1.50	30.35	NS
Lot 25	OmyRGT33TUF	Ogo7UW	UnDef	37.25	$P < 0.05$

[a] Indicates the female:male recombination difference for the pair of markers indicated in each respective mapping family. UnDef indicates 0 recombination in the male for the specified interval.
[b] The average centimorgan (θ) distance on the linkage group between the pair of markers indicated.
[c] Results from a contingency G-test (1 d.f.) comparing parental *vs.* recombinant classes between the sexes.

In addition, the expanded male recombination interval between OmyRGT23TUF and OmyRGT21TUF on chromosome 8 suggests a telomeric position for these two markers that is supported by gene-centromere distances estimates (*i.e.*, 43.4 and 45.7 cM, respectively; unpublished data). Male recombination levels in telomeric regions are not necessarily higher than those of the female in all linkage groups (*i.e.*, linkage group H; Figure 1 and Table 2). The recombination ratio in telomeric regions is estimated to be 0.14:1 (female:male ratio), while regions proximal to the centromere have an ~10:1 (female:male) recombination ratio (Figure 2 and Table 2).

Distribution of duplicated SSR markers: Several of

the SSR markers had four alleles instead of two. It was possible to score these duplications as disomically segregating (Allendorf and Danzmann 1997) in the female parent and, in most cases, the male parent. The distribution of the pairs of disomic markers helped to identify chromosome arms that might show pseudolinkage arrangements (*i.e.*, homeology to one another during meiosis in the male). For example, OmyFGT8/iiTUF, One18/iiASC, and Omy272/iiUoG are linked on chromosome 5, while OmyFGT8/iTUF, One18/iASC, and Omy272/iUoG are linked on chromosome 15. The linkage of these SSR markers to allozyme markers *sIDHP-3** and *mIDHP-2** confirms the earlier pseudolinkage arrangement (*i.e.*, pseudolinkage group V) reported by May and Johnson (1990).

We have used the conservation of duplicated SSR markers to tentatively identify additional pseudolinkage arrangements. In linkage groups Oi and Oii, six duplicated marker pairs have been detected and their pseudolinkage has been confirmed by the pseudolinkage of OmyFGT32/iiTUF to OmyFGT32/iTUF. Additive gene distances place OmyFGT32/iTUF and OmyFGT32/iiTUF at the telomeres. Linkage group Oii also shows linkage affinities to linkage group 2 (LOD, 2.0 threshold). This and the fact that SmaBFRO1/i and SmaBFRO1/ii are shared between these two linkage groups suggests that one arm of linkage group Oii may be pseudolinked to chromosome 2 [*i.e.*, a possible affinity of Oii to pseudolinkage group I of May and Johnson (1990)]. One14ASC in linkage group O has a gene-centromere distance of 49 cM (P. Howard, unpublished data) and is linked to Oi in the male map but not the female map. Thus, One14ASC may represent a terminal marker on a translocation arm to linkage group Oi or the marker may be located in a telomeric position in pseudolinkage group Oii. Marker *TRCARR*/i(INRA) may also possess similar arrangements, but we are unable to ascertain its linkage with One14ASC since segregation data were obtained from separate families. The chromosome arm containing *TRCARR*/i(INRA) may show homeology to linkage group T, which possesses *TRCARR*/ii(INRA) in lot 25. Linkage groups Fi and Fii share three duplicated marker regions and secondary tetrasomy is confirmed by the pseudolinkage of OmyRT10/iTUF to OmyRT10/iiTUF in the male (Figure 1).

Other duplicated marker pairs were identified on the various linkage groups, but we were unable to ascertain their actual pseudolinkage arrangements since both markers were not polymorphic in the same male parent or were unlinked in the male. Nevertheless, many duplicated markers may not show evidence of pseudolinkage if they are located more proximal to the centromere, as was observed for pseudolinkage group O, or if they are telomeric and occur close to chiasmata junctions (see model presented in discussion). Linkage groups tentatively identified as showing some homeology to one another because of the presence of duplicated markers are chromosomes A and K (Ogo2/iUW and Ogo2/iiUW, OmyFGT21/iTUF and OmyFGT21/iiTUF), chromosomes C and L (OmyRGT6/iiTUF and OmyRGT6/iTUF), chromosomes D and T (Omy296/iiUoG and Omy296/iUoG); chromosomes H and U (OmyCosB/iiTUF and OmyCosB/iTUF), and, possibly, linkage group G to Q (Omy27/iINRA and Omy27/iiINRA) although the affinity of Omy27/iINRA to either arm of linkage group G is uncertain.

Sex-determining locus: We found two SSR markers (OmyFGT19TUF and OmyRGT28TUF) linked to the sex-determining locus in males (Figure 1). OmyFGT19TUF is more closely linked to sex (LOD = 23.82, θ = 1.15; lot 44) than OmyRGT28TUF. OmyFGT19TUF is also closely linked to the allozyme locus *bGLUA-2** (average θ = 1.2 among several half-sib families not used in this study), supporting the previous report of sex-linkage with this allozyme locus (Allendorf *et al.* 1994). The estimated gene-centromere distance for OmyFGT19TUF was 2.04 (P. Howard, unpublished data), placing the sex locus close to centromere as previously reported (May and Johnson 1990). We also report the more distal linkage of an SSR marker (Omy17INRA) to linkage group 18. This marker shows zero recombination with the allozyme marker *sSOD-1** in the female parent of lot 25. *sSOD-1** was previously reported to be sex linked in rainbow trout, but is telomeric in location on linkage group 18L and thus is a weak marker for sex (Allendorf *et al.* 1986, 1994).

Differences in the recombination rates between two families: We compared the recombination rates for the same pairs of markers shared between the female parents of lot 25 and lot 44 across 10 linkage groups. Recombination ratios between lot 44:lot 25 varied from 0.345:1 to 4.858:1 (Table 3) and appeared higher in the lot 44 female (P = 0.06; Wilcoxon paired-sample test).

DISCUSSION

Sex-specific recombination rates: In human, mouse, cattle, and pig, and indeed in most vertebrates studied thus far, recombination rates show significant differences between the sexes. Female map distances are usually greater than those in the male (Barendse *et al.* 1994; Ellegren *et al.* 1994; Dib *et al.* 1996; Dietrich *et al.* 1996). Ratios (F:M) average from approximate unity to 1.8:1 within many species including humans (Gyapay *et al.* 1994; Archibald *et al.* 1995; Mellersh *et al.* 1997; Knapik *et al.* 1998). Birds may show a reversal of this trend, with slightly higher rates in males compared with females (Groenen *et al.* 1998), which appears consistent with Haldane's prediction (Haldane 1922) that the heterogametic sex shows slightly lower recombination rates.

Recombination rates in male salmonids are also repressed relative to females, presumably because of structural constraints imposed on crossing over within multivalent pairings. Such pairings often involve meta-

TABLE 3

Ratio of observed recombination differences in females between pairs of markers among various linkage groups in lot 25 and lot 44

Linkage group	Marker region	Segregation ratio Lot 44:Lot 25
5	One18/iiASC–Omy272/iiUoG	0.345
15	One18/iASC–Omy272/iUoG	2.051
8	OmyRGT21TUF–OmyRGT23TUF	1.878
A	OmyRGT41TUF–OmyFGT5TUF	0.625
B	Omy301UoG–Ssa197DU	1.379
G	Ssa85DU–OmyRGT36TUF	1.162
	OmyRGT36TUF–One2ASC	0.767
	One2ASC–OmyFGT16TUF	1.798
N	OmyFGT28/iiTUF–OmyRGT32TUF	0.928
	OmyRGT32TUF–OmyRGT47TUF	0.587
Oi	OmyRGT30TUF–OmyRGT33TUF	4.858
	OmyRGT33TUF–OmyFGT18/iTUF	3.245
	OmyFGT18/iTUF–OmyFGT32/iTUF	1.991
Oii	OmyFGT18/iiTUF–OmyFGT32/iiTUF	4.620
R	One1/iASC–OmyFGT26TUF	0.971

centric chromosomes resulting from Robertsonian fusions of ancient acrocentric chromosomes, which in turn may pair with their respective acrocentric homeologues (Lee and Wright 1981; Wright *et al.* 1983; Johnson *et al.* 1987; Jackson *et al.* 1998). We were able to assess recombination rate differences between the sexes and between individuals by using outcrossed families and gene-centromere mapping approaches (Thorgaard *et al.* 1983) to localize centromeres within linkage groups. Using this approach, we report the largest sex-specific recombination differences for any known vertebrate. These findings are consistent with previous reports of large female:male recombination differences among salmonid species detected with allozymes (Johnson *et al.* 1987).

The unusually large recombination differences between males and females are postulated to arise from the differential chromosome pairing affinities observed between the sexes. Multivalents are often formed during meiosis (due to the tetraploid ancestry of these fishes), but these formations appear almost exclusively in males (Wright *et al.* 1983; Allendorf and Thorgaard 1984). As a consequence, large recombination rate differences may arise between the sexes and may be conditional upon the chromosomal localization of chiasmata (Figure 3). If chiasmata are localized to telomeric regions (Wright *et al.* 1983; Allendorf and Danzmann 1997), then regions proximal to the centromere may experience no crossing over in the male, while telomeric regions may experience an exchange of genetic material with homeologous regions. This would tend to inflate the recombination levels in the telomeric regions of males compared to females.

The hybrid nature of the males used as our mapping parents likely enhances the large sex-specific segregation differences we observed. Hybrids possess balanced sets of chromosomes derived from either founder strain after their formation. However, when these individuals form gametes, unbalanced chromosome associations may be evident. Hybrid genomic incompatibilities have previously been proposed to account for the high de-

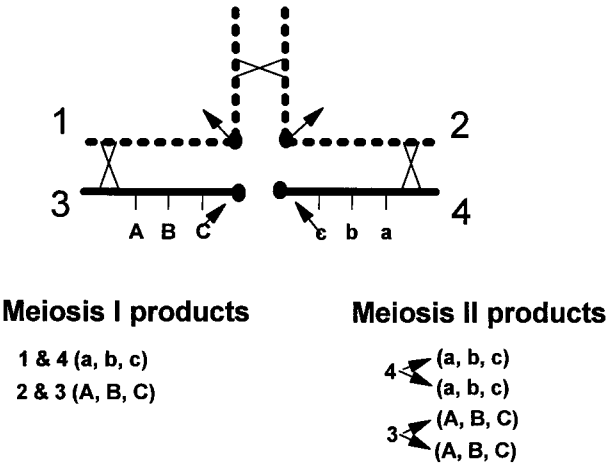

Figure 3.—Multivalent formations occurring in males that may account for a lack of recombination among linked markers proximal to centromeric positions. Homeologous arms are numbered 1–4 and pair with each other distally following homologous pairing of chromosome arms. Robertsonian translocations may give rise to nonhomeologous fusions. During quadrivalent formation, homeologous pairings of chromosomes occur with crossovers restricted to telomeric regions. At meiosis I, chromosomes involved in crossing over migrate to opposite poles (arrows) such that chromosome 1 and 4 segregate together, as do chromosomes 2 and 3 (*i.e.*, alternate disjunction). Following segregation at meiosis II only intact (noncrossover) chromosomal segments proximal to the centromere are transmitted in bivalent set 3,4. Thus, the parental phase of all the markers in this region will not be disrupted, giving the appearance of tight linkage over a large chromosomal distance (adapted from Wright *et al.* 1983).

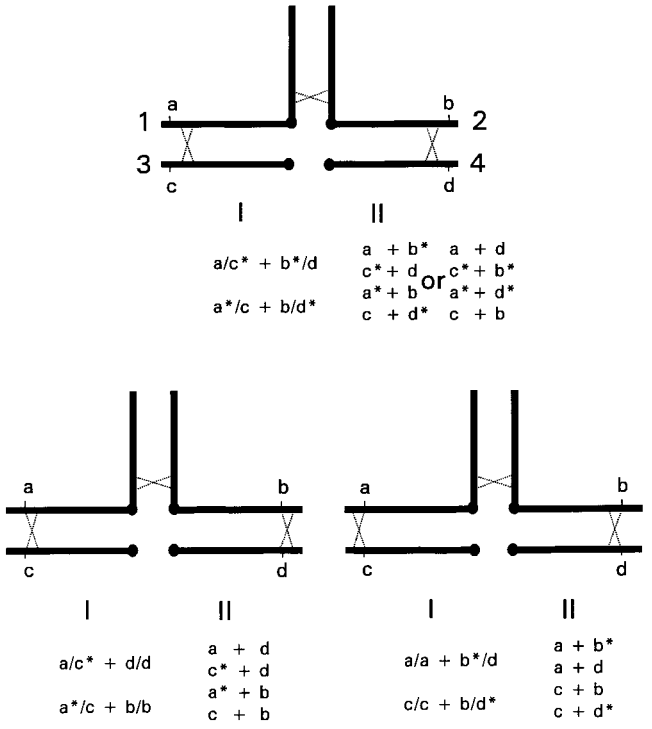

Figure 4.—Gamete formation expected in males at duplicated marker sets located on homeologous chromosome sets that form multivalents during meiosis I. The top shows how an excess of recombinant genotypes (pseudolinkage) are produced by duplicated markers derived from homeologous multivalent sets. The genotypes of the duplicated telomeric loci are a/b for the locus located on the metacentric homeologues (locus i) and c/d for the locus located on the acrocentric homeologues (locus ii). Only a single chromatid strand is shown; however, the crossover chromatid segments generated during meiosis I are depicted with an asterisk, such that allele a resides on the noncrossover chromatid of chromosome 1 while allele c* is derived from a crossover chromatid. Assuming alternate disjunction of homeologues following meiosis I, chromosome 1 would segregate with chromosome 4, and 2 with 3, such that a/c* and b*/d alleles (on chromosomes 1 and 4, respectively) would migrate to one pole, while a*/c and b/d* alleles (on chromosomes 2 and 3, respectively) would go to the opposite pole. Exclusive formation of recombinant genotypes could occur only if crossover chromatids paired with noncrossover chromatids, or pairing was exclusive within crossover and noncrossover chromatids. Following the pairing of crossover with noncrossover chromatid segments at meiosis II, only a + b* and c* + d gametes derived from chromosomes 1 and 4 and a* + b plus c + d* gametes derived from chromosomes 2 and 3 would result. If pairing was exclusive within noncrossover and crossover chromatids, only a + d and c* + b* gametes would result from the alternate disjunction of chromosomes 1 and 4, while a* + d* and c + b gametes would result from the alternate disjunction of chromosomes 2 and 3. If chiasmata formation was unequal with respect to the position of a duplicated subtelomeric marker (bottom), only one of the two duplicated markers would exchange terminal chromatid segments for the marker region of interest, while noncrossover chromatids would segregate at meiosis I for the other duplicated marker. This would be interpreted as random segregation of the markers (see text for explanation).

gree of pseudolinkage observed in male salmonid hybrids (Wright *et al.* 1983). A decrease in pseudolinkage observed in subsequent backcross generations involving such hybrids is also thought to be related to the process of increasing genomic compatibility as chromosomal segments are exchanged in each subsequent backcross generation (Davisson *et al.* 1973; Wright *et al.* 1983).

Male meiosis: Wright *et al.* (1983) proposed a model to account for the secondary tetrasomy or pseudolinkage observed in male salmonids. Essentially, this model proposes that multivalent chromosome pairings in males are formed by metacentric chromosomes involving Robertsonian fusions of two nonhomeologous acrocentric pairs. Subsequent to the formation of such metacentrics, homeologous acrocentric pairs of chromosomes may randomly pair with their homeologous arms in the metacentrics to form multivalents at meiosis. In an F_1 hybrid, two types of pairings are possible: the acrocentric and metacentric chromosome derived from the same parent could pair together or homeologous acrocentrics derived from the father could pair with the homeologous metacentric arm derived from the mother, and vice versa. Wright *et al.* (1980, 1983) proposed that the former type of pairing primarily occurs (*i.e.*, the same sex parental homeologues pair). Furthermore, separation of chromosomes from multivalent formations was proposed to be primarily of an alternate nature (*i.e.*, see Figure 3, where chromosome 1 and 4 would always go to one pole while chromosomes 2 and 3 would go to the other). Alternate disjunction accounts for the excess of nonparental genotypes generated at duplicated loci in some males and also accounts for the lack of double-reduction gametes (identical alleles derived from the same locus pairing after meiosis II) observed in males. Double-reduction gametes are only expected following adjacent chromosome segregation from multivalents (Burnham 1962). This model was extended by Allendorf and Thorgaard (1984) to account for all types of chromosome pairings in hybrid salmonid males. They proposed that it was only necessary for one pair of homeologous chromosomes to pair (derived from the same parent) to initiate multivalent formations. Prior to such homeologous pairings it was predicted that homologous pairing would be initiated close to the centromere in the other multivalent arm of the metacentric. Separation of homeologously paired chromosomes to opposite poles following meiosis I would also ensure that an excess of recombinant genotypes were formed, which is characteristic of pseudolinkage [consistent with the Wright *et al.* (1983) model, assuming the pairing of crossover with noncrossover chromatids or that only crossover and noncrossover chromatids pair at meiosis II—see Figure 4].

The models outlining the mechanics of chromosome segregation in male salmonids suggest that if multivalent formations occur in a relatively high frequency during

male meiosis, many of the genes located closer to the centromere may appear to segregate as a block, since crossover events will be localized toward the telomeres. Even if intrahomologue pairings are initiated close to the centromere (Allendorf and Thorgaard model) the regions distal to such crossover locations will segregate as a block between the centromeric and telomeric crossover junctions. The current data comparing male and female distances clearly indicate that map distances are greatly reduced in males for regions surrounding the centromere, while they appear to be greatly expanded in certain linkage groups for markers that appear to be telomeric according to gene-centromere map distances.

Pseudolinked markers appear linked in the male because recombinants are primarily formed following meiosis. This balance in the formation of alleles results because only nonancestrals (derived from different parental chromosomes) appear to pair following meiosis I. This will occur for any set of markers along a linkage group that are proximal or distal to the location of chiasmata formation. However, for markers that experience a crossover event proximal to the position of the marker on one homeologue and distal to the position of the marker on the other homeologue, it is expected that such markers may in fact appear unlinked in males (see Figure 4). In pseudolinkage group F, for example, the terminal markers (*i.e.*, Omy3/iINRA and Omy3/iiINRA) appear unlinked in the male (see Figure 1). We believe this may be explained by the localization of chiasmata formation in this region of the homeologues leading to an unbalanced segregation of homeologue segments within this region.

To explain this phenomenon, assume that a duplicated marker had the following genotypes (*i.e.*, a/b at locus i and c/d at locus ii) and the ancestrally derived homeologues (*i.e.*, homeologues inherited from the same parent) were a/c and b/d. A crossover event proximal to the a/c paired homeologues would result in a/c and c/a chromatids, while b/b and d/d chromatids would result if the chiasmata formed distal to this region in the other paired homeologues. Following alternate disjunction of the homeologues and separation of chromatids at meiosis II, ad, cd gametes would be formed by chromosomes 1 and 4, while ab, cb gametes would be formed by disjunction of chromosomes 2 and 3 (Figure 4). Similarly, if crossover events occurred proximal to the marker in the b/d paired homeologues, but distal to the a/c paired homeologues (*i.e.*, the reciprocal situation), the following gametes would result: cb, cd gametes from alternate disjunction of chromosomes 2 and 3 and ab, ad from alternate disjunction of chromosomes 1 and 4 (Figure 4). Thus, from either type of unequal crossover event identical gametes would result. Since the gametic vectors resulting from such unequal crossover events would be ab:ad:cb:cd in a 1:1:1:1 ratio, it would be inferred that the male parental genotype was a/c at locus i and b/d at locus ii. However, as shown, such gametic vectors may in fact result from male genotypes that are a/b at locus i and c/d at locus ii. Thus, without prior knowledge of the parental phase of the alleles at the duplicated markers, the true genotypes of the parental markers would be incorrectly inferred.

Assuming the above dynamics, it is possible that duplicated markers occurring in regions of localized chiasmata formation in multivalents would appear unlinked in the male parent. Such a process could account for the lack of linkage for the duplicated markers Omy3/iINRA and Omy3/iiINRA in the male parent we used in lot 25. Duplicated markers that appear unlinked in male salmonids may provide information on chromosome arm regions where chiasmata are localized. This would be supported by future linkage data from females that localize such markers toward telomeric regions of a chromosome arm.

Recombination differences between families: The differences in recombination rates we detected between lots 25 and 44 were unexpected and may be related to whether these chromosomal regions show evidence of secondary tetrasomic associations (*i.e.*, pseudolinkage). Extreme differences in recombination rates between families were observed with linkage groups Oi and Oii, as well as linkage groups 5 and 15. Linkage groups 5 and 15 were reported to be pseudolinked by May and Johnson (1990) and the microsatellite data obtained from this study supports this finding. Recombination differences detected in other linkage groups were not as great, although telomeric regions may show greater differences. Large differences in recombination rates between the female parents of our two main mapping families were found for telomeric marker pairs on linkage groups N, G, and 8 (Table 3). Since the process of secondary tetrasomic exchange in males will alter the degree of homology along chromosomal segments, descendents of given males may possess differential affinities among homologous chromosomal segments (Allendorf and Danzmann 1997). Consequently, variation in recombination rate may be related to the degree of homology among chromosomal segments within individuals. Those segments that are more closely related may show a greater degree of crossing over.

Segregation distortion: Two marker regions (One14-ASC in linkage group Oi and One1/iiASC in linkage group R) showed significant segregation distortion ($P < 0.005$) in one (One14ASC in lot 25) or both of the female parents (One1/iiASC in lots 25 and 44) used. These regions are of interest because they are unassigned in the female map, but linked in the male map. Thus, the two regions may represent terminal markers on the designated linkage groups or they may identify separate chromosomes showing residual tetrasomy with the specified linkage groups. The degree of segregation distortion could account for their lack of assignment to a specified linkage group.

The rainbow trout map: The variation in recombination rates between the sexes observed in the present study may partially explain the distribution of AFLP markers observed by Young *et al.* (1998; *i.e.*, an apparent clustering of AFLP markers into centromeric regions in the rainbow trout map). If multivalent formations were prevalent in the F_1 parent (derived from androgenic haploid lines) used in their testcross, then many of the reported AFLP markers may not in fact be as tightly linked in females (*i.e.*, due to lack of recombination in male genomes). The suggestion that crossing over may generally be suppressed in centromeric regions has previously been proposed (Keim *et al.* 1997) and is supported by experimental findings that suggest a suppression of crossover in regions of heterochromatin (Choo 1998). Our evidence suggests that crossover suppression may extend over large reaches of the comparative female map (perhaps 40 cM or greater on each arm) but primarily results from chromosome pairing dynamics in salmonids rather than physical properties within the chromosomes. Terminal markers in certain linkage groups in the Young *et al.* (1998) map may have greatly expanded map distances since our data suggest that crossover events are localized within the terminal regions of each chromosome arm in the male.

Future research: Our findings suggest that the parental origins of chromosomes involved in meiotic pairings may have a very large influence on the resulting gametic vector of alleles. This appears true for both male and female parents, although the results are more pronounced in males used in this study presumably due to their hybrid origin. The influence of hybrid genome background on gamete formation may be investigated in the future by using half-sib brothers of hybrid and nonhybrid origin.

Differences in recombination rates observed between our female mapping parents were not consistent across all chromosomal regions, which may also reflect upon the degree of homology between the female chromosomes undergoing meiosis. Both female mapping parents were derived from lines that had been undergoing recombination for only seven (lot 25) to five generations (lot 44). The commercial line (from which the lot 44 female was obtained) was initially started from one founding female and fewer than 10 founding males. While the exact parental origin of the other strain giving rise to the lot 25 female is not known (it was derived from a thermal selection experiment involving over 2500 fish from three diverse genetic sources in the initial generation (Ihssen 1986), it is believed that a greater number of individuals contributed genetic material to this line. The higher recombination rates generally detected in lot 44 compared to the lot 25 female may indicate that chromosomal compatabilities are higher in the genomic background of this individual. Future assessment of the influence of chromosomal coancestry on recombination rates in salmonids would be of interest.

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